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Dr Daninel Darley University of Marburg, Germany, 1 July 2002–12 July 2002

Scope of Research

Structure and function of biocatalysts, in particular, pyridoxal enzymes and enzymes acting on xenobiotic compounds, are studied to elucidate the dynamic aspects of the fine mechanism for their catalysis in the light of recent advances in gene technology, protein engineering and crystallography. In addition, the metabolism and biofunction of sulfur, selenium, and some other trace elements are investigated. Development and application of new biomolecular functions of microorganisms are also studied to open the door to new fields of biotechnology. For example, molecular structures and functions of psychrophilic enzymes and their application are under investigation.

Research Activities (Year 2002)

Presentations

Studies of the function of selenium in mouse brain, Kuwana E, Mihara H, Esaki N, 2002 Annual Meeting, Jpn. Soc. Biosci, Biotech, and Agrochem., 26 March.

Iron-sulfur cluster formation in *E.coli*: Properties of the IscS-IscU complex, Kato S, Mihara H, Kurihara T, Yoshimura T, Esaki N. 2002 Annual Meeting, Jpn. Soc. Biosci, Biotech, and Agrochem., 26 March.

Identification of the proteins involved in 2-chroloacryliric acid metabolism in *Burkholderia* sp., Kurata A, Kurihara T, Kamachi H, Esaki N, 2002 Annual Meeting, Jpn. Biochem. Soc., 16 October.

Properties of *N*-methyl-L-amino acid dehydrogenase of *Pseudomonas putida*, Kakutani R, Mihara H, Yasuda M, Ueda M, Esaki N, 2002 Annual Meeting, Jpn. Biochem. Soc., 17 October.

Grants

Esaki N, Structural biology and biosynthesis of selenium-containing proteins, Grant-in-Aid for Scientific Research (B), 1 April 2001 - 31 March 2003.

Esaki N, Construction and functional analysis of composite biocatalysts, Grant-in-Aid for Scientific Reseach on Priority Areas (B), 1 April 2001 - 31 March 2004.

Esaki N, Determination of whole genome sequence of psychrophilic bacteria, analysis of genes involved in their adaptation to cold environments, and exploitation of cold-active enzymes, Grant-in-Aid for Scientific Research (B)1 April 2001 - 31 March 2003.

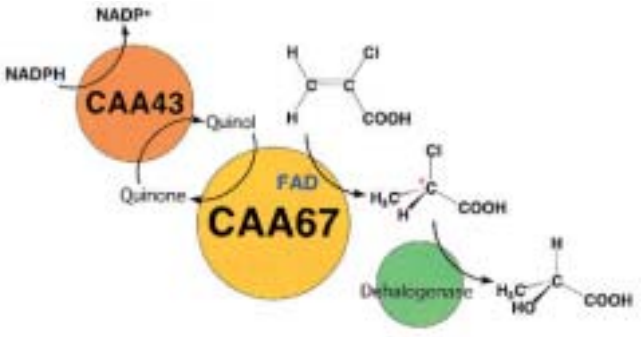
Mihara H, Analyses of *suf* gene cluster and Suf proteins involved in iron transport, Grant-in-Aid for Young Scientists B, 1 April 2001 - 31 March 2003.

Yoshimura T, Physiological role of D-amino acids in eukaryote, Grant-in-Aid for Scientific Reseach (C), 1 April 2002 - 31 March 2004.

Kurihara T, Bioconversion of fluorinated organic compounds: catalytic mechanisms of elimination and incorporation of fluorine and their application, Grant-in-Aid

A Novel Enzyme Catalyzing Asymmetric Reduction of Carbon-Carbon Double Bond

Asymmetric reduction of carbon-carbon double bonds is one of the most useful methods for production of chiral compounds. We have isolated a novel bacterium, *Burkholderia* sp. WS, catalyzing asymmetric reduction of 2-chloroacrylic acid to produce *S*-2-chloropropionic acid, which is the essential building block of aryloxyphenoxypropanoic acid, the herbicide skeleton most widely used for broadleaf crops such as cotton and soybean. In bacterium, 2-chloroacrylic acid is converted into *S*-2-chloropropionic acid, which is subsequently hydrolyzed to lactic acid by the action of 2-haloacid dehalogenase. The activity of 2-chloroacrylic acid reductase was induced when the bacterium was grown on 2-chloroacrylic acid as the carbon source, and we found that three proteins were inducibly synthesized in accordance with the induction of the reductase activity. One of them was supported to be 2-haloacid dehalogenase, and other two, named CAA67 and CAA43, were thought to participate in the reduction of 2-chloroacrylic acid. The genes coding for CAA67 and CAA43 were cloned and sequenced. The two genes constituted a gene cluster. CAA67 and CAA 43 showed sequence similarity to fumarate reductase and NAD(P)H: quinone oxidoreductase, respectively. CAA 67 probably catalyzes reduction of 2-chloroacrylic acid, and CAA43 most likely functions as a supplier of reducing power for CAA67.

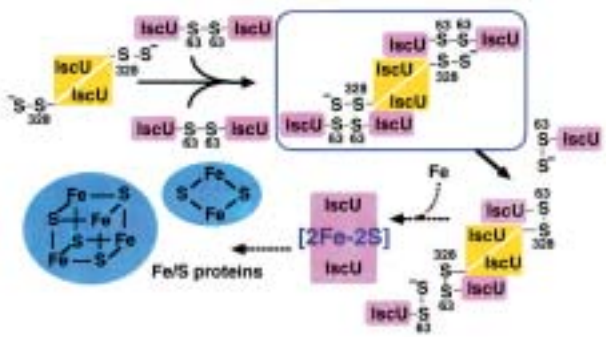


Scheme 1. Probable assimilation pathway of 2-chloroacrylic acid in *Burkholderia* sp. WS

Assembly of Iron-Sulfur Cluster in *Escherichia coli*

Iron-sulfur proteins are widely distributed in organisms and play essential roles in energy metabolism, DNA repair, transcriptional regulation, and biosynthesis of nucleotides and amino acids. Recent studies demonstrated that the assembly of their prosthetic groups, iron-sulfur clusters, is mediated by proteins encoded by the *isc* operon in prokaryotes and by their counterparts in eukaryotes. Among these proteins, IscS catalyzes the desulfurization of L-cysteine and cooperates with IscU in the biosynthesis of iron-sulfur clusters in *E. coli*. IscS and IscU form a covalent complex, and a sulfur atom derived from L-cysteine is transferred from IscS to IscU. We found that the disulfide bond is formed between Cys328 of IscS and Cys63 of IscU. We also found that Cys 63 of IscU is essential for the IscU-mediated activation of IscS.

Based on the findings, we propose a mechanism for an early stage of iron-sulfur cluster assembly: the sulfur transfer from IscS to IscU is initiated by the attack of Cys63 of IscU on the Sγ atom of IscS-Cys328 that is bound to sulfane sulfur derived from L-cysteine.



Scheme 2. Formation of iron-sulfur clusters

for Young Scientists A, 1 April 2002 - 31 March 2004.

Yoshimura T, Construction of Enzymes with new functions by loop engineering, Seed Research Program of Biotechnology, Ministry of Agriculture, Forestry, Fisheries of Japan, 1 April 2000 - 31 March 2005.

Kurihara T, Production of useful compounds and bioremediation of environments by cryobiotechnology using cold-adapted microorganisms, (NEDO), 1 April 2001 - 31 March 2004.

Kurihara T, *In vivo* and *in vitro* analysis of selenium metabolism - a multidisciplinary approach, Cooperative Research under the Japan-U.S. Cooperative Science Program(JSPS) 1 April 2001 - 31 March 2004.